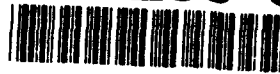


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CONTRACT NO.: DAMD17-90-C-0131

TITLE: STUDY OF COMPOUNDS FOR ACTIVITY AGAINST LEISHMANIA.

PRINCIPAL INVESTIGATOR: William L. Hanson, Ph.D.

PI ADDRESS: Department of Parasitology
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REPORT DATE:

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; Distribution unlimited.

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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 27/04/94	3. REPORT TYPE AND DATES COVERED FINAL REPORT (28/09/90 - 27/03/94)		
4. TITLE AND SUBTITLE STUDY OF COMPOUNDS FOR ACTIVITY AGAINST <u>LEISHMANIA</u>		5. FUNDING NUMBERS Contract No. DAMD17-90-C-0131		
6. AUTHOR(S) Dr. William L. Hanson, Principal Investigator Dr. J. Roger Broderon, Co-Principal Investigator Dr. Willie L. Chapman, Jr., Co-Principal Investigator Ms. Virginia B. Waits				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Georgia Research Foundation, Inc. Boyd Graduate Studies Research Center (GSRC) The University of Georgia Athens, GA 30602-7411		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U. S. Army Medical Research and Development Command Fort Detrick Frederick, MD 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; Distribution unlimited		12b. DISTRIBUTION CODE		
13. ABSTRACT (Maximum 200 words) During this contract, a total of 205 selected compounds were studied for efficacy against <u>Leishmania</u> <u>Leishmania donovani</u> infections in hamsters. Forty-four of these compounds were active as indicated by 50 percent or greater parasite suppression. Glucantime® Indices ranged from 1.418 to 0.351. Among these active compounds were 8-aminoquinolines (which are the most efficacious), phenanthrene methanols, dibenzopyrroles, disulfides, aminothiols, and C-nucleosides. When Glucantime®, Pentostam® or Amphotericin B were given in combination with the 8-aminoquinoline, WR06026, antileishmanial efficacy was not enhanced significantly over that observed with WR06026 alone. A total of 146 compounds were studied for efficacy against <u>Leishmania</u> <u>Viannia braziliensis</u> and 42 were active as indicated by 50 percent or greater suppression of lesion area caused by these parasites. Glucantime® Indices for these active compounds ranged from 40.9 to 0.474. Generally, the most active compounds were 8-aminoquinolines. Many of these compounds were active when administered either orally or via the intramuscular routes. In addition, a phosphonium compound had some efficacy against <u>Leishmania</u> (V.) <u>braziliensis</u> as did Sinefungin. Both of these compounds are toxic. A total of 51 selected oligonucleotides were studied <u>in vitro</u> for antileishmanial activity against promastigotes of <u>Leishmania</u> (L.) <u>donovani</u> . Only one of these was noted to be active.				
14. SUBJECT TERMS <u>Leishmania</u> <u>Leishmania donovani</u> ; <u>Leishmania</u> <u>Viannia braziliensis</u> ; Chemotherapy; Sinefungin; Phosphoniums; 8-aminoquinolines; Oligonucleotides; Phenanthrene Methanols; Trifluralin; Dibenzopyrroles		15. NUMBER OF PAGES 36		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

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TABLE OF CONTENTS

Foreword.....	1
Introduction.....	3
Materials and Methods.....	4
I. Visceral Test System.....	4
A. Primary Test System.....	4
B. Studies Involving the Extension of the Treatment Regimen.....	4
C. Combination Studies.....	5
II. Cutaneous Test System.....	5
A. Primary Test System.....	5
B. Studies Involving the Extension of the Treatment Regimen.....	5
C. Studies Involving the Extension of Time for Lesion Measurements.....	6
III. Comparative Antileishmanial Activity of Selected Compounds Against <i>L. (L.) donovani</i> and <i>L. (V.) braziliensis</i>	6
IV. <i>In vitro</i> Studies of Oligonucleotides Against <i>L. (L.) donovani</i>	6
Results.....	7
I. Visceral Test System.....	7
A. Primary Test System.....	7
B. Studies Involving the Extension of the Treatment Regimen.....	7
C. Combination Studies.....	7
II. Cutaneous Test System.....	7
A. Primary Test System.....	7
B. Studies Involving the Extension of the Treatment Regimen.....	7
C. Studies Involving the Extension of Time for Lesion Measurement.....	7
III. Comparative Antileishmanial Activity of Selected Compounds Against <i>Leishmania Leishmania donovani</i> and <i>Leishmania Viannia braziliensis</i>	7
IV. <i>In vitro</i> Studies of Oligonucleotides Against <i>Leishmania Leishmania donovani</i>	9
Discussion.....	10
Conclusions.....	12
Literature Cited.....	13
Appendix.....	14
Distribution List.....	36

INTRODUCTION

Protozoan parasites of the genus *Leishmania* are widespread throughout the world where they cause a complex of visceral or cutaneous diseases in human beings as well as some animals including dogs in numerous tropical and sub-tropical countries (1,2,3). Since the leishmaniasis commonly exist as zoonoses, these diseases pose a significant potential threat to military personnel as well as military dogs throughout endemic areas. Relatively recent publicity regarding infection of personnel involved in Operation Desert Storm has reemphasized the military significance of the leishmaniasis.

Better drugs are needed for the treatment of the leishmaniasis since those currently available are often not satisfactorily effective and are potentially toxic to man and animals.

This laboratory has been involved for several years in studies to identify new compounds for antileishmanial activity against both visceral (*Leishmania Leishmania donovani*) and cutaneous (*Leishmania Viannia braziliensis*) leishmaniasis. Although several new compounds have been identified with activity against *L. (V.) braziliensis*, none have shown adequate promise to warrant initiation of clinical trials. However, among the most promising active compounds found against visceral leishmaniasis during these studies is the 8-aminoquinoline, WR06026 (4). This compound is now undergoing clinical trials in Kenyan visceral leishmaniasis patients. Screening for compounds active against visceral leishmaniasis has continued during this project period in the event that WR06026 does not perform in the field as expected and screening has continued to identify more active and less toxic compounds for *L. (V.) braziliensis*.

This report summarizes the results of studies conducted for this contract during the period September 28, 1990 through March 27, 1994.

MATERIALS AND METHODS

I. Visceral Test System

A. Primary Test System

A Khartoum strain of *L. (L.) donovani* (WR378) was used and the golden hamster (*Mesocricetus auratus*), 50-70 gm, served as the host animal. Suspensions of amastigotes for infection of experimental hamsters were prepared by grinding heavily infected hamster spleens in sterile saline in a Ten Broeck tissue grinder and diluting the suspensions so that 0.2 ml contained approximately 10×10^6 amastigotes. Each experimental hamster was infected via the intracardiac injection of 0.2 ml of the amastigote suspension.

The testing procedure used was that described by Stauber and his associates (5,6,7) as modified by Hanson, et al. (8). On Day 3 following infection, hamsters were divided randomly into experimental groups consisting of a minimum of 6 animals per group, initial group weights were obtained, and administration of test compounds was initiated. Each compound was tested at 2 or 3 drug dosage levels dependent on the priority rating and nature of the compound.

The vehicle for the test compounds was 0.5% hydroxyethyl cellulose-0.1% Tween 80 (HEC-Tween). Each test group contained six hamsters and received one of the desired drug dosage levels. A control group of six hamsters received the 0.5% HEC-Tween vehicle only and the reference compound, Glucantime®, was given at 3 drug dosage levels (208, 52, and 26 total mg/kg) based on antimony content. All test compounds were administered routinely twice daily via the intramuscular route on Days 3 through 6. Final group weights were obtained on all experimental hamsters on Day 7 and all animals were killed, livers removed, weighed, and liver impressions made for enumeration of amastigotes. Subsequently, the total number of parasites per liver was determined as described by Stauber, et al. (5,6,7).

In addition to recording body weight changes as a general indicator of toxicity of the test compounds, experimental hamsters were observed for such clinical signs of toxicity as nervous disorders, roughened hair coat, and sluggish activity. Deaths of the animals was also considered indicative of significant drug toxicity.

After determining the ratio of numbers of amastigotes per host cell nucleus the weight of the organ, and initial and final weights of the hamsters, the raw data was evaluated with an IBM PC XT microcomputer using a program which calculates percent weight change, total numbers of parasites, mean numbers of parasites per organ, and percent parasite suppression. The computer program then performs linear and non-linear regression analysis and calculates a SD_{50} for active compound from each of the analyses (drug dosage resulting in 50% suppression of amastigotes). The SD_{50} from the non-linear analysis is used for a comparison of the relative efficacy of the test compounds and the efficacy of test compounds relative to that of the reference compound, Glucantime®. The linear regression analysis is included only for comparison with the non-linear analysis.

B. Studies Involving the Extension of the Treatment Regimen

The testing procedures used in these studies were the same as those used for the primary visceral test system above with the exception of the treatment regimen. Hamsters were treated twice daily beginning on

Day 3 postinfection for a total of 10 working days (Monday through Friday, Monday through Friday). Final group weights were taken, animals killed, and liver impressions made on Day 17 postinfection.

C. Combination Studies

Studies were conducted in which Glucantime® (BL09186), Pentostam® (BL06916), and Amphotericin B (BM16033) were administered to groups of hamsters in combination with WR06026 (BK01845) using the most efficacious route of administration for each compound. Treatment was begun on Day 3 postinfection and was given in a single injection. The four drugs were also administered alone to groups of hamsters to serve as controls. All hamsters were killed and liver impressions made on Day 7 postinfection for enumeration of parasite burdens.

II. Cutaneous Test System

A. Primary Test System

Leishmania (V.) braziliensis (WR539) was used in these studies. Male golden hamsters, 50-70 gm, served as experimental hosts.

Promastigotes for establishing experimental infections in hamsters were grown in Schneider's Drosophila Medium (Hendricks, et al., 9) and quantitated using procedures described previously (Hanson and Roberson, 10). In preparation for infection and weekly during the experiment, the hair was clipped on the dorsal tail head and a commercial depilatory agent applied to the areas to remove the remaining hair. Each hamster was inoculated via the intradermal route with approximately 1.5×10^7 promastigotes of *L. (V.) braziliensis* near the base of the tail using a 0.25 ml glass syringe equipped with a 30 gauge X 1/2" needle. Each experimental group consisted of six hamsters. Initial body weights were obtained and administration of therapy, generally via the intramuscular route, was initiated on Day 19 postinfection, and continued through Day 22 postinfection. Glucantime® was included at two dosage levels (832 and 208 total mg/Sb/kg) as the reference compound, and a group of six hamsters received vehicle only (HEC-Tween). Test compounds were administered generally at 416 and 208 total mg/kg.

Lesion area of each experimental hamster was determined one week after completion of treatment with the aid of a template made at WRAIR and calibrated according to the formula $r_1 r_2 \pi$ where r_1 is the major radius of the lesion and r_2 is the minor radius (Wilson, et al., 11). The mean lesion area of each experimental group was obtained and the percent suppression of lesion size calculated by comparing the mean lesion area of each treated group with that of the group receiving vehicle only with the aid of a computer program and an IBM PC XT microcomputer. The computer program performs linear and non-linear regression analysis and calculates an SD_{50} for each active compound using both analyses. The SD_{50} obtained from the non-linear analyses is used for a rough comparison of the relative efficacies of the test compounds and the relative efficacy of test compounds with that of the reference compound, Glucantime®. The linear regression analysis is performed for comparison with the non-linear analysis.

B. Studies Involving the Extension of the Treatment Regimen

The procedures used in these studies were the same as those used for the primary cutaneous test system with the exception that the treatment regimen was increased from four days to 10 days. Lesions were measured one week following completion of treatment on Day 37 postinfection.

C. Studies Involving the Extension of Time for Lesions Measurements

One experiment was conducted during this contract in which cutaneous lesions were measured at one, two, three, four, six and eight weeks following completion of treatment (Day 78 postinfection). Procedures relating to infection of animals, treatment, and analysis of data were the same as those used for the primary cutaneous test system.

III. Comparative Antileishmanial Activity of Selected Compounds Against *L. (L.) donovani* and *L. (V.) braziliensis*.

The most active compounds in the primary cutaneous test system were selected for these studies from the data base. Thirty-seven compounds were tested simultaneously in the primary cutaneous and primary visceral test systems using the procedures described above in sections IA and IIA. Since most of the active compounds were 8-aminoquinolines, two routes of administration (*i.e.*, intramuscular and oral) were used against each parasite. Dosage levels for the cutaneous test system were generally higher than that used for the visceral test system due to the fact that the reference compound, Glucantime®, requires approximately a four-fold higher dosage level in the cutaneous system than in the visceral test system for activity.

IV. *In vitro* Studies of Oligonucleotides Against *L. (L.) donovani*

Promastigotes of *L. (L.) donovani* were cultured from an infected hamster spleen in Schneider's Drosophila Medium (Hendricks, et al., 9) and quantitated using procedures described previously (Hanson and Roberson, 10). Promastigotes from four-day cultures (fourth to twelfth subpassage) were used in this work. (Unpublished data indicates that this age culture is the best for establishing infections in hamsters.)

Cultures were harvested by centrifugation and resulting pellets were resuspended in Schneider's Drosophila Medium to a final concentration of 6.5×10^5 per ml. Using round bottom microtiter plates (Dynatech), 200 μ l of the parasite suspension was added to each well and plates incubated at 26°C (Day 0).

Approximately 24 hours later, the oligonucleotides were added to appropriate wells at 30 micromolar concentrations (Day 1). Sets of four cultures were used for each, as well as for untreated controls. Cultures were again incubated until Day 4, at which time total numbers of promastigotes/ml for each well were determined using the procedures described by Hanson and Roberson (10).

Mean numbers of parasites per well for each treated well and for untreated wells were calculated. Percent suppression or inhibition of parasite growth was determined using the following formula:

Percent Suppression = mean number of parasites for the untreated controls minus the mean number of parasites for the test compound divided by the mean number of parasites for the untreated control times 100.

Negative percent suppression indicated enhanced growth of parasites in the treated wells as compared to growth in the untreated wells.

RESULTS

I. Visceral Test System

A. Primary Test System

During this contract period, a total of 205 compounds were studied for efficacy against *Leishmania (L.) donovani* infections in hamsters (Table I). Forty-four of these compounds were active, as indicated by 50 percent or greater parasite suppressions. Glucantime® Indices ranged from 1418 (BM16033) to 0.351 (BE20274). Among these active compounds were 8-aminoquinolines, phenanthrene methanols, dibenzopyrroles, C-nucleosides, disulfides, and aminothiols.

B. Studies Involving the Extension of the Treatment Regimen

Nine compounds (Table II) were studied for suppressive activity against *L. (L.) donovani* by extending the treatment period from four days to ten days. None of these compounds were active as tested.

C. Combination Studies

When Glucantime®, Pentostam®, or Amphotericin B were given in combination with either dosage level of WR06026, suppressive activity was not enhanced significantly over that observed with WR06026 alone (Table III).

II. Cutaneous Test System

A. Primary Test System

One-hundred forty-six compounds were studied for efficacy against *L. (V.) braziliensis* in the primary cutaneous test system (Table IV). Forty-two of these were active as indicated by 50 percent or greater reduction of parasitic lesion area. Glucantime® Indices for these active compounds ranged from 40.9 (AH07870) to 0.474 (ZP47054).

B. Studies Involving the Extension of the Treatment Regimen

Three compounds were administered for 10 days, rather than four days, to hamsters. Two of these compounds (BM17316 and BM17325) were given topically while the third (BM15876) was administered orally. None of these compounds were active as indicated by less than 50 percent reduction of lesion area.

C. Studies Involving the Extension of Time for Lesion Measurement

Nine compounds were administered to groups of hamsters and final lesion measurements taken eight weeks following completion of treatment in order to allow additional time for the compounds to decrease lesion size. Four of these compounds were active (Table V) at eight weeks posttreatment while six were active at one week posttreatment (the time used for the primary screen).

III. Comparative Antileishmanial Activity of Selected Compounds Against *L. (L.) donovani* and *L. (V.) braziliensis*

A group of compounds which were selected from the cutaneous test system data base because they had been found to have antileishmanial activity equal to or greater than the reference compound, Glucantime®, were studied simultaneously via the oral and intramuscular routes for .

efficacy against both *L. (L.) donovani* and *L. (V.) braziliensis* for comparative purposes as well as to determine the compound most active against *L. (V.) braziliensis* as indicated in Table VI.

It was noted that, with two exceptions (WR049577 and WR027794), those compounds that were active at all against *L. (V.) braziliensis* were considerably more active against *L. (L.) donovani*. For example, four 8-aminoquinoline compounds (WR211789, WR211666, WR223658, WR223756) were 99-100% suppressive against *L. (L.) donovani* at the lowest dosage level tests (either 6.5 or 13 mg/kg) when administered either orally or via the intramuscular route. Additional studies (Table IV) were done on these compounds to determine the SD_{50} for comparative purposes. In contrast, only WR211789 and WR223658 were active against *L. (V.) braziliensis*, and the SD_{50} 's of each of these compounds were in excess of 100 mg/kg against this parasite. All of these compounds except WR211789 showed evidence of toxicity to hamsters when administered at dosage levels of 104 or 208 mg/kg against *L. (V.) braziliensis*.

Ten of the 37 compounds studied in these experiments were sufficiently active against *L. (V.) braziliensis* to be of interest. Eight of these were 8-aminoquinoline compounds. Among these, WR006007 with an SD_{50} of 79.8 mg/kg was approximately seven times less efficacious against *L. (V.) braziliensis* than against *L. (L.) donovani* when administered via the intramuscular route. Similar comparative studies using the oral route of administration could not be done because of the insufficient quantity of this compound available. WR027794 was approximately 3-4 times less potent against *L. (V.) braziliensis* than *L. (L.) donovani* and this compound appeared to be equally effective when administered via the oral or intramuscular routes. Similarly the efficacy of WR027779 was approximately two-fold more active against *L. (L.) donovani* and this compound was approximately equally active when administered orally or intramuscularly. The difference in potency of WR027780 against *L. (V.) braziliensis* and *L. (L.) donovani* was likewise approximately two-fold but this compound was about twice as active when administered via the intramuscular route than via the oral route. The efficacy of both WR006877 and WR006021 was two to three times greater against *L. (L.) donovani* than against *L. (V.) braziliensis*. Although the activity of these compounds against *L. (L.) donovani* was similar when administered either orally or intramuscularly, these compounds were active against *L. (V.) braziliensis* only when administered via the intramuscular route. WR006881 (SD_{50} = 77.7 mg/kg) was only slightly less potent against *L. (V.) braziliensis* than *L. (L.) donovani*.

The most active compound against *L. (V.) braziliensis* was the 8-aminoquinoline, WR049577 (SD_{50} = 3.76 mg/kg). Although this compound was the most potent compound studied against *L. (V.) braziliensis*, it was not active when administered orally and is toxic (causing weight loss in recipient hamsters) at dosage levels as low as 26 mg/kg while suppressing lesion size by only 76% at this same dosage.

Regarding the two active compounds that were not 8-aminoquinoline, one (WR122536) was a phosphonium compound which had an SD_{50} of 58 mg/kg when administered via the intramuscular route. Unfortunately, this compound was toxic (caused weight loss in recipient hamsters) at 104 mg/kg.

The other active compound that was not an 8-aminoquinoline was Sinefungin (WR254847). This compound has been tested previously in this laboratory and found to be active against both *L. (V.) braziliensis* and *L. (L.) donovani* in hamsters (see Final Report, Contract No. DAMD17-85-C-5012, October 31, 1990). The difference in the activity of this

compound against *L. (V.) braziliensis* and *L. (L.) donovani* was greater in the current experiments than in initial studies.

IV. *In vitro* Studies of Oligonucleotides Against *L. (L.) donovani*

Table VII summarizes the results of the *in vitro* testing of 51 selected oligonucleotides for inhibition of growth of promastigotes of *L. (L.) donovani*. One oligonucleotide (LE001.01J 910806) appeared to suppress the multiplication of *L. (L.) donovani* in two separate studies (91.4% and 53.0% inhibition). Due to the differences in percent suppression obtained in the two experiments, a confirmatory experiment would have been desirable before drawing a final conclusion on the activity of this compound.

DISCUSSION

The 8-aminoquinoline, WR06026, is the most promising antileishmanial compound identified in this laboratory to date and is currently undergoing some phases of clinical trials for treatment of visceral leishmaniasis. In the event that this compound does not perform in clinical trials as hoped, work has continued in this laboratory to identify other promising antileishmanial drugs for both visceral and cutaneous leishmaniasis. To this end, several hundred selected compounds representing 8-aminoquinolines, phenanthrene methanols, dibenzopyrroles, disulfides, aminothols, antitubulins, and others were studied during this project period for activity against both visceral and cutaneous leishmaniasis in hamsters. In addition, the duration of treatment, route of administration of treatment, as well as selected drug combinations were studied.

As verified by these studies, the 8-aminoquinolines are the most active compounds against both *Leishmania (L.) donovani* and *Leishmania (V.) panamensis* in hamsters. Furthermore, as verified by these studies, the 8-aminoquinolines generally are more active against *L. (L.) donovani* than against *L. (V.) panamensis*. The reference compound, Glucantime®, is also more active against *L. (L.) donovani* than *L. (V.) panamensis*.

The reasons for the higher efficacy of the 8-aminoquinolines against *L. (L.) donovani* are unknown, but it may be due to the fact that liver parasites are more accessible to the parent compounds and their metabolites since this class of compounds are metabolized in the liver. Apparently, less compound and/or metabolites is distributed to sites distant to the liver, a hypothesis supported by observations in this laboratory of less activity of these compounds against splenic parasites than liver parasites in *L. (L.) donovani* infections in hamsters.

This suggested problem of bioavailability appears to be an especially important one in cutaneous leishmaniasis. It is possible that this question could be addressed by regimen variation or possibly application of the drug directly onto the lesion.

When test compounds were compared for efficacy against both visceral and cutaneous leishmaniasis in the same experiment, no clear pattern emerged regarding the relationship of route of administration to activity. Some compounds were more active when administered orally while others were more active when administered via the intramuscular route.

Thirteen C-nucleosides had been previously tested, but none was more active than was formycin-B. These compounds have been noted to be extremely toxic to the host. The absence of additional compounds of this class in the inventory resulted in the decision to cease further examination of C-nucleosides for the immediate future, although compounds of this class remain of long-term interest.

The phenanthrene methanol, WR149809 (AX64884), had previously shown antileishmanial activity, while other phenanthrene methanols have shown antimalarial activity in rodents or primates. The three antimalarial phenanthrene methanols (AY91608, AX63172, AX67009) tested in our system failed to show significant antileishmanial activity. The lack of potency of these compounds combined with possible toxicity at higher dose levels has led us to conclude that further investigation of this class of compounds should be limited to any available phenanthrene methanols with an alkyl side chain similar to that of WR06026.

Although the herbicide, trifluralin, has been reported to have antileishmanial activity *in vitro*, neither this compound nor any of its

analogues tested in our system showed *in vivo* activity. Such a disparity between *in vitro* and *in vivo* antiparasitic activity is not surprising and does not invalidate using reported cases in *in vitro* activity as leads for drug testing. It is anticipated that such leads will continue to be exploited in the future, in particular in instances where compounds involved represent new classes of potential drugs, e.g., natural product derivatives.

Antisense RNA's have been exploited with varying success to block the activity of specific genes to inhibit the replication of viruses as well as various human cancer cells (12, 13). Dr. R. Meyer, Microprobe, Inc., under a separate contract (DAMD17-88-C-8201) developed the idea to apply this technology against *Leishmania* and has synthesized a number of antisense as well as sense oligonucleotides for possible inhibition of the growth of *Leishmania*. These oligonucleotides were supplied to our laboratory for testing. Thus far, this approach has not appeared to be especially promising although some suggestion of inhibition of growth of *Leishmania (L.) donovani* *in vitro* was observed in one or two instances. One possible explanation for the lack of inhibition observed in these experiments is the fact that it is sometimes difficult to get the oligonucleotides into cells at the right time to block messenger RNA activities (12).

When hamsters infected with *L. (L.) donovani* were treated with WR06026 plus amphotericin B, WR06026 plus Pentostam®, or WR06026 plus Glucantime®, no enhancement of the activity of WR06026 was achieved in these experiments. Based on these data, no advantage either in parasite suppression or possible cure of infection is achieved with these drug combinations.

CONCLUSIONS

1. The 8-aminoquinolines are the most active antileishmanial compounds studied to date against both *Leishmania donovani* and *Leishmania Viannia braziliensis*.
2. Generally, the 8-aminoquinolines as well as Glucantime® are more active against *L. (L.) donovani* than *L. (V.) panamensis*.
3. Studies comparing the oral and intramuscular routes of administration of promising antileishmanial compounds revealed no clear pattern since some compounds are more active when administered orally, some are more active when administered via the intramuscular route, and the activity of some is similar when either route is used.
4. The C-nucleosides, trifluralin, phenanthrene methanols, and antisense nucleotides were not particularly promising antileishmanial candidates in these studies due to either lack of potency or toxicity to the host.
5. Combining WR06026 with either amphotericin B, Glucantime®, or Pentostam® did not enhance the antileishmanial activity of this 8-aminoquinoline.
6. The primary and visceral test systems used in this laboratory provide accurate and dependable evaluation of potential antileishmanial compounds since extension of dosing regimen or time to evaluation of lesion size did not alter the results obtained during the standard procedures.

LITERATURE CITED

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Appendix

Table I. Summary of the suppressive activity of selected compounds against *Leishmania donovani* in the hamster.

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
BE20354	3.25	16	13	64	52	83	9.84	2.58
BG56256	0.05	26	0.2	2	0.8	46	NDND	NDND
BH50802	0.8	23	3.25	53	13	98	2.52	10.0
BH72317	0.2	- 3	0.8	26	3.25	74	1.61	15.7
BL20649	.80	17	3.25	22	13	45	NDND	NDND
AX64884	13	17	104	43	416	31	NDND	NDND
BH67432	13	100	52	100	3025	98	3.47	14.7
ZN41968	13	9	52	50	208	99	99.6	.513
ZN42812	.05	20	.2	16	.8	13	NDND	NDND
ZP39981	.05	- 12	.2	- 7	.8	- 1	NDND	NDND
ZP40699	.2	18	.8	14	3.25	42	NDND	NDND
ZP40868	.8	13	1.6	16	3.25	13	NDND	NDND
BJ04583	13	- 7	52	0	208	44	NDND	NDND
BE20274	26	- 20	104	21	416	86	241.	.351
BJ04636	13	- 15	52	30	104	29	NDND	NDND
BK74375	13	- 23	52	16	104	41	NDND	NDND
AE73324	13	21	52	- 2	208	3	NDND	NDND
AS72596	13	8	52	19	208	13	NDND	NDND
AU14450	13	15	52	16	208	23	NDND	NDND
AX17250	13	16	52	9	208	26	NDND	NDND
ZP41749	13	- 17	52	- 3	208	39	NDND	NDND
AY14763	13	- 18	52	- 38	208	- 30	NDND	NDND
BE75019	13	- 43	52	- 15	208	- 1	NDND	NDND
BK13925	13	- 27	52	- 38	208	- 19	NDND	NDND
BE20943	52	51	104	80	208	100	50.9	3.02
AU59100	52	78	104	90	208	100	32.7	4.70
AG09379	52	1	208	- 50	NDNDN	NDN	NDND	NDND
AG33437	52	- 43	208	- 30	NDNDN	NDN	NDND	NDND
AL73520	52	- 29	208	- 5	NDNDN	NDN	NDND	NDND
AM10000	52	18	208	1	NDNDN	NDN	NDND	NDND
AM10162	52	10	208	22	NDNDN	NDN	NDND	NDND
AT51074	52	- 12	208	3	NDNDN	NDN	NDND	NDND
ZE67962	52	2	208	4	NDNDN	NDN	NDND	NDND
ZE96721	52	9	208	18	NDNDN	NDN	NDND	NDND
ZN66045	52	5	208	- 14	NDNDN	NDN	NDND	NDND
BH72317	0.20	- 3	0.80	- 4	3.25	16	NDND	NDND
BL20649	0.80	6	3.25	- 1	13	- 11	NDND	NDND
ZN44003	0.1	- 2	0.4	19	1.6	5	NDND	NDND
ZP25914	0.20	- 6	0.80	- 1	3.25	12	NDND	NDND
ZP40868	0.20	- 3	0.80	14	3.25	20	NDND	NDND
AX64884	52	20	208	16	832	25	NDND	NDND
BE20274	52	- 8	104	19	208	61	178.	.738
BE20532	52	1	104	4	208	6	NDND	NDND
BE20943	52	68	104	85	208	100	35.9	2.53
BJ04583	52	41	104	65	208	56	71.2	1.28
BK74375	52	51	104	81	208	82	50.4	1.80
ZN41968	0.8	- 7	3.25	13	13	21	NDND	NDND
AH02786	52	- 7	208	- 5	NDNDN	NDN	NDND	NDND
BM08808	52	- 12	208	- 14	NDNDN	NDN	NDND	NDND

Table I. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
AX76053	13	25	52	29	104	14	NDND	NDND
AX76062	13	32	52	29	104	42	NDND	NDND
AY91608	13	27	52	28	104	37	NDND	NDND
AX63172	13	17	52	26	104	43	NDND	NDND
AX67009	13	- 24	52	5	104	- 5	NDND	D
AQ38003	26	- 1	52	- 5	208	- 8	NDND	D
BC10527	26	- 22	52	13	208	- 8	NDND	D
BG46894	52	- 5	208	- 2	NDNDN	NDN	NDND	D
BC02936	52	- 13	416	9	NDNDN	NDN	NDND	NDND
BC86165	52	- 29	416	5	NDNDN	NDN	NDND	NDND
BK03947	52	- 19	416	1	NDNDN	NDN	NDND	NDND
BK13774	52	- 19	416	- 16	NDNDN	NDN	NDND	NDND
BK13989	52	- 12	416	- 15	NDNDN	NDN	NDND	NDND
BK14011	52	7	416	- 1	NDNDN	NDN	NDND	NDND
AX26820	26	- 18	52	- 28	104	21	NDND	NDND
AX26820	26	- 24	52	- 32	104	- 20	NDND	NDND
BK50713	6.5	99	13	100	52	100	2.14	85.0
BK50713	6.5	99	13	100	52	100	1.98	91.8
BG11417	6.5	100	13	100	52	100	1.25	145.
BG11417	6.5	100	13	100	52	100	1.44	125.
AG78374	6.5	- 2	13	2	52	61	1.56	116.
AG98545	13	- 18	52	67	104	NDN	41.7	2.58
AG98545	13	18	52	51	104	86	45.7	2.35
BJ92403	13	17	52	91	104	100	24.3	4.43
BJ92403	13	- 20	52	62	104	98	47.0	2.29
BG21744	13	100	52	100	104	100	3.73	28.8
BG21744	13	100	52	100	104	100	3.59	30.0
AH16404	6.5	4	13	9	26	9	NDND	NDND
BLO9186	52	23	104	45	208	56	150.	0
BG22125	13	100	52	100	104	100	4.19	0
BG22125	13	100	52	100	104	100	3.47	0
AH07870	6.5	14	13	26	26	34	NDND	D
AH07870	6.5	- 27	13	- 16	26	- 30	NDND	D
AJ09575	13	- 4	52	1	104	84	82.3	0
AJ09575	13	10	52	25	104	48	NDND	D
BM10620	13	29	52	18	104	38	NDND	NDND
BM10620	13	17	52	22	104	- 6	NDND	NDND
BM10620	13	18	52	27	104	19	NDND	NDND
BE20532	6.5	3	13	- 1	52	0	NDND	NDND
BE20532	6.5	- 4	13	14	52	10	NDND	NDND
AJ36812	13	54	52	90	104	98	11.9	13.5
BB18813	13	11	52	56	104	99	49.4	3.97
BB18813	13	- 36	52	60	104	98	47.6	4.12
BB19758	13	- 14	26	- 24	104	- 25	NDND	NDND
BB19758	13	- 22	26	- 27	104	- 17	NDND	NDND
BE20112	13	99	26	99	104	100	4.93	39.8
BE20112	13	99	26	100	104	100	4.65	42.2
BE20318	13	- 17	26	- 5	104	11	NDND	NDND
BE20318	13	- 35	26	- 11	104	5	NDND	NDND
BE20345	13	- 11	26	2	104	4	NDND	NDND
BE20345	13	16	26	50	104	55	92.0	2.01
BE20354	6.5	15	13	50	52	77	26.9	6.88
BE20354	6.5	26	13	28	52	77	29.9	6.21

Table I. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
AH32668	13	- 13	52	2	104	- 15	NDND	NDND
BE20498	13	22	26	30	104	84	53.2	3.61
BE20498	13	20	26	40	104	68	52.8	3.64
BE20792	6.5	25	26	13	52	46	NDND	NDND
BE20792	6.5	21	26	28	52	20	NDND	NDND
BE20925	6.5	10	26	44	52	82	28.7	6.68
BE20925	6.5	16	26	33	52	60	41.6	4.61
ZN29695	6.5	- 37	13	17	52	72	35.6	2.40
ZN29695	6.5	- 25	13	- 13	52	76	38.2	2.24
BE21039	6.5	17	26	61	52	75	20.7	4.13
BE21039	6.5	9	26	58	52	72	23.6	3.62
BE21066	6.5	7	26	15	52	57	23.6	3.62
BE21066	6.5	1	26	14	52	47	NDND	NDND
BE21084	6.5	- 1	26	65	52	83	18.8	8.65
BE21084	6.5	- 5	26	26	52	58	44.1	3.70
BE21511	6.5	- 41	13	- 24	52	14	NDND	NDND
BE21511	6.5	- 7	13	1	52	26	NDND	NDND
BE21799	6.5	9	13	3	52	78	34.8	4.69
BE21799	6.5	3	13	- 5	52	61	34.8	4.69
BL05848	6.5	74	13	93	52	99	4.16	19.8
BL05848	6.5	64	13	82	52	98	4.47	18.5
AJ09851	13	16	52	40	104	44	NDND	NDND
BE20603	6.5	29	13	35	52	78	24.6	3.36
BE20943	6.5	26	13	27	52	52	48.5	1.70
BM10371	52	37	208	25	NDNDN	NDN	NDND	NDND
AT63681	13	3	52	22	104	- 9	NDND	NDND
BL58705	6.5	75	26	89	52	85	1.71	39.7
AJ15304	13	7	26	22	52	34	NDND	NDND
AT56097	13	13	52	51	104	38	51.4	1.32
BL21100	52	14	208	17	NDNDN	NDN	NDND	NDND
AY97173	52	33	208	36	NDNDN	NDN	NDND	NDND
AY97315	52	35	208	10	NDNDN	NDN	NDND	NDND
BL29759	52	24	208	25	NDNDN	NDN	NDND	NDND
BL34170	52	2	208	- 5	NDNDN	NDN	NDND	NDND
BL56390	13	- 12	52	- 34	208	- 18	NDND	NDND
BL59588	52	- 42	208	- 44	NDNDN	NDN	NDND	NDND
AX26839	52	19	208	- 3	NDNDN	NDN	NDND	NDND
AH90393	52	33	208	16	NDNDN	NDN	NDND	NDND
AD60466	52	8	208	3	NDNDN	NDN	NDND	NDND
AN35100	52	36	208	6	NDNDN	NDN	NDND	NDND
AP64866	52	12	208	20	NDNDN	NDN	NDND	NDND
AG50330	52	0	208	- 1	NDNDN	NDN	NDND	NDND
AN15359	52	19	208	9	NDNDN	NDN	NDND	NDND
BM12991	52	- 7	208	- 14	NDNDN	NDN	NDND	NDND
AG66089	52	12	208	12	NDNDN	NDN	NDND	NDND
AR81714	52	23	208	40	NDNDN	NDN	NDND	NDND
BM12491	13	1	52	7	208	- 10	NDND	NDND
BM12491	52	- 13	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BM12508	13	- 3	52	- 6	208	0	NDND	NDND
BM12508	52	11	NDNDN	NDN	NDNDN	NDN	NDND	NDND
AR94417	52	36	208	28	NDNDN	NDN	NDND	NDND
ZP10397	52	51	208	NDN	NDNDN	NDN	50.9	1.45

Table I. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
AE95204	52	- 13	208	22	NDNDN	NDN	NDND	NDND
AJ91813	52	- 19	208	4	NDNDN	NDN	NDND	NDND
AM04315	52	- 12	208	- 11	NDNDN	NDN	NDND	NDND
AN39528	52	13	208	35	NDNDN	NDN	NDND	NDND
AQ07393	52	7	208	28	NDNDN	NDN	NDND	NDND
AS64898	52	- 21	208	- 17	NDNDN	NDN	NDND	NDND
BL86558	52	- 19	208	- 9	NDNDN	NDN	NDND	NDND
ZA01419	52	- 20	208	15	NDNDN	NDN	NDND	NDND
ZC07751	52	- 15	208	0	NDNDN	NDN	NDND	NDND
ZC07760	52	3	208	- 4	NDNDN	NDN	NDND	NDND
ZG81239	52	1	208	16	NDNDN	NDN	NDND	NDND
AP86979	52	- 7	208	- 4	NDNDN	NDN	NDND	NDND
AR02802	52	- 10	208	- 3	NDNDN	NDN	NDND	NDND
AH69718	52	8	208	- 23	NDNDN	NDN	NDND	NDND
AL02996	52	22	208	13	NDNDN	NDN	NDND	NDND
AG53831	52	27	208	22	NDNDN	NDN	NDND	NDND
AG53859	52	- 12	208	14	NDNDN	NDN	NDND	NDND
AG53840	52	16	208	25	NDNDN	NDN	NDND	NDND
BE16494	89	- 8	356	- 2	NDNDN	NDN	NDND	NDND
AJ32190	89	- 9	356	- 11	NDNDN	NDN	NDND	NDND
AH95665	89	13	356	18	NDNDN	NDN	NDND	NDND
BE99420	89	- 40	356	1	NDNDN	NDN	NDND	NDND
ZB27758	89	- 10	356	- 18	NDNDN	NDN	NDND	NDND
AF55410	89	- 8	356	5	NDNDN	NDN	NDND	NDND
BG01377	89	1	356	1	NDNDN	NDN	NDND	NDND
BJ52052	89	- 7	356	17	NDNDN	NDN	NDND	NDND
BJ52043	89	21	356	27	NDNDN	NDN	NDND	NDND
ZB27990	89	- 3	356	23	NDNDN	NDN	NDND	NDND
AJ02030	89	28	356	26	NDNDN	NDN	NDND	NDND
ZB27641	89	- 5	356	16	NDNDN	NDN	NDND	NDND
BM15527	52	4	208	4	832	28	NDND	NDND
BM15527	0.8	15	3.25	5	13	- 4	NDND	NDND
BM15518	13	7	52	23	208	4	NDND	NDND
BM15509	52	30	208	12	832	- 7	NDND	NDND
BL29446	52	- 21	208	0	NDNDN	NDN	NDND	NDND
BL06916	52	35	104	63	416	99	76.6	2.60
BM16033	0.16	71	0.5	94	1.5	99	.140	1418
BK01845	0.36	65	1.08	97	3.25	100	.289	688.
BK01845	0.12	10	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BM15509	600	- 38	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BM15518	600	- 70	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BM15527	600	- 19	NDNDN	NDN	NDNDN	NDN	NDND	NDND
ZN48083	600	- 44	NDNDN	NDN	NDNDN	NDN	NDND	NDND
AQ97922	400	NDN	NDNDN	NDN	NDNDN	NDN	NDND	NDND
AY27939	400	- 62	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL06916	26	10	52	34	208	60	147.	1.35
BM18395	208	56	416	92	832	92	185.	.748
BM18402	52	- 15	832	18	NDNDN	NDN	NDND	NDND

Table I. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
AMO	52	9	208	23	ND	ND	ND	ND
BP	52	12	208	20	ND	ND	ND	ND
CPZ	52	23	208	16	ND	ND	ND	ND
HLP	52	8	208	23	ND	ND	ND	ND
PR	52	10	208	34	ND	ND	ND	ND
BL20649	.8	17	3.25	22	13	45	ND	ND
AX64884	13	17	104	43	416	31	ND	ND
BH67432	3.25	98	13	100	52	100	NC	NC
ZN41968	13	9	52	50	208	99	NC	NC
ZN42812	.05	20	.2	16	.8	13	ND	ND
BE20354	.2	- 48	.8	- 37	3.25	- 39	ND	ND
BG56256	.1	- 16	.4	- 29	1.6	82	NC	NC
BH50802	.2	- 28	.8	- 28	3.25	32	ND	ND
BH67432	.1	- 17	.4	1	1.6	96	NC	NC
BK01845	.4	32	.8	94	3.25	99	NC	NC
BK01845	.4	1	.8	78	3.25	99	NC	NC
BK50713	.4	- 23	.8	- 6	3.25	97	NC	NC
BK50713	.4	- 29	.8	6	3.25	94	NC	NC
BG11417	.4	29	.8	59	3.25	99	NC	NC
BG11417	.4	28	.8	68	3.25	99	NC	NC
BK01845	.4	7	.8	82	3.25	100	NC	NC
BK01845	.4	18	.8	80	3.25	100	NC	NC
BG21744	.4	- 31	.8	12	3.25	100	NC	NC
BG21744	.4	- 5	.8	25	3.25	99	NC	NC
BG22125	.4	- 16	.8	42	3.25	98	NC	NC
BG22125	.4	6	.8	31	3.25	94	NC	NC
BK01845	.4	0	.8	77	3.25	100	NC	NC
BK01845	.4	30	.8	77	3.25	100	NC	NC
BE20112	.4	8	.8	18	3.25	51	NC	NC
BE20112	.4	- 13	.8	8	3.25	55	NC	NC
BD29263	52	- 10	208	- 44	ND	ND	ND	ND
BD29165	52	20	208	54	ND	ND	NC	NC
BC82407	52	24	208	37	ND	ND	ND	ND
BL06916	26	77	52	83	208	90	NC	NC

Table II. Summary of the suppressive activity of selected compounds against *Leishmania donovani* when administered for ten days duration.

<u>BN</u>	<u>Dose1</u>	<u>Suppres1</u>	<u>Dose2</u>	<u>Suppres2</u>	<u>Dose3</u>	<u>Suppres3</u>	<u>SD₅₀</u>	<u>GI</u>
BM16613	400	23	ND	ND	ND	ND	ND	ND
BM16604	190	17	ND	ND	ND	ND	ND	ND
BM16622	400	22	ND	ND	ND	ND	ND	ND
BM17905	180	7	ND	ND	ND	ND	ND	ND
BM17898	200	0	400	16	800	19	ND	ND
BM17889	400	-9	ND	ND	ND	ND	ND	ND
BM17870	400	-7	ND	ND	ND	ND	ND	ND
BL20934	100	9	400	23	800	-9	ND	ND
BL20934	52	14	208	-8	416	4	ND	ND

Table III. Summary of the suppressive activity of Glucantime®, Pentostam®, Amphotericin B, and WR06026 alone and in combination with WR06026 Against *Leishmania donovani* in the hamster.

Compound	TMG	ALONE		+ .2 WR06026		+ .4 WR06026	
		% Supp.	SD ₅₀ *	% Supp.	SD ₅₀	% Supp.	SD ₅₀
Glucantime	208	71	149	91	90	98	42
BL09186	104	31		63		93	
Pentostam	160	88	97	99	82	98	55
BL06916	80	31		41		94	
Amphotericin B	.4	88	.19	96	.18	98	.17
BM16033	.2	58		72		77	
WR06026	.4	94	.19	Not Done		Not Done	
BK01845	.2	63					

*non-linear

Table IV. Summary of the suppressive activity of selected compounds against *Leishmania (V.) braziliensis* in the hamster.

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
BM10620	13	7	52	51	104	NDN	51.0	3.02
BM10620	13	- 7	52	- 7	104	11	NDND	NDND
BM10620	13	- 59	52	- 11	104	15	NDND	NDND
AH07870	6.5	61	13	61	26	76	3.76	40.9
AH07870	6.5	0	13	- 7	26	- 3	NDND	NDND
AJ09575	26	24	104	11	208	19	NDND	NDND
AJ09575	26	- 30	104	15	208	15	NDND	NDND
BLO9186	208	64	832	91	NDNDN	NDN	161.	0
BE20532	13	7	52	11	104	11	NDND	D
BB19758	26	- 25	104	- 2	208	- 7	NDND	D
BB18813	52	- 7	104	19	208	- 7	NDND	D
BB18813	52	- 21	104	- 7	208	7	NDND	D
BB19758	26	- 4	104	0	208	11	NDND	D
BE20532	13	- 14	52	- 7	104	- 14	NDND	D
BE20112	26	21	104	48	208	NDN	NDND	NDND
BE20112	26	28	104	17	208	NDN	NDND	NDND
BE20318	26	14	104	5	208	NDN	NDND	NDND
BE20318	26	7	104	7	208	- 3	NDND	NDND
BE20345	26	21	104	37	208	NDN	NDND	NDND
BE20345	26	28	104	- 3	208	14	NDND	NDND
BE20354	13	30	52	47	104	67	59.3	6.21
BE20354	13	7	52	44	104	72	61.0	6.03
BE20498	26	37	104	46	208	60	133.	2.76
BE20498	26	- 15	104	15	208	53	199.	1.84
BE20792	6.5	2	26	42	104	54	77.7	4.74
BE20792	6.5	- 19	26	- 26	104	- 30	NDND	NDND
AH16404	6.5	0	13	- 4	26	33	NDND	NDND
BE21039	13	25	52	71	208	81	33.7	5.48
BE20925	6.5	17	26	13	104	38	NDND	NDND
ZN29695	13	13	52	41	104	71	65.0	2.85
ZN29695	13	- 17	52	17	104	43	NDND	NDND
BE20925	6.5	10	26	53	104	78	25.9	7.14
BE21039	13	20	52	57	208	75	44.3	4.17
AJ36812	13	7	52	37	104	61	79.8	2.32
BE21066	13	16	52	42	104	32	NDND	NDND
BE21066	13	- 10	52	10	104	19	NDND	NDND
BE21084	26	23	52	52	104	79	50.1	3.98
BE21084	26	- 23	52	19	104	55	96.3	2.07
BE21511	13	- 13	26	0	104	6	NDND	NDND
BE21511	13	6	26	- 6	104	3	NDND	NDND
AH32668	52	0	104	3	208	- 3	NDND	NDND
BE21799	13	- 12	26	- 8	104	55	606.	1
BE21799	13	- 4	26	8	104	42	NDND	NDND
BL05848	13	15	52	32	104	NDN	NDND	NDND
BL05848	13	- 19	52	17	104	NDN	NDND	NDND
AJ09851	26	- 19	104	- 4	208	53	201.	3.01
BL58705	13	35	52	43	104	56	79.6	2.24
BE20603	13	- 9	52	4	104	26	NDND	NDND
BE20943	13	- 35	52	4	104	58	96.1	1.86

Table IV. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
AT63681	52	- 15	104	- 13	208	- 13	NDND	NDND
AJ15304	3.25	- 9	13	- 30	52	9	NDND	NDND
AT56097	52	9	104	- 4	208	13	NDND	NDND
BK01845	1.6	15	6.5	4	13	30	NDND	NDND
BK01845	0.4	- 4	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BK01845	1.6	11	6.5	15	13	26	NDND	NDND
BK01845	0.4	26	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL58705	13	60	52	61	104	61	10.7	26.9
BL58705	13	22	52	11	104	33	NDND	NDND
BH67432	1.6	11	6.5	15	13	19	NDND	NDND
BH67432	0.4	7	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL34296	1.6	4	6.5	- 4	13	4	NDND	NDND
BL34296	0.4	- 4	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL52196	1.6	- 13	6.5	- 22	13	- 13	NDND	NDND
BL52196	0.4	- 9	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL53308	1.6	- 9	6.5	- 22	13	- 9	NDND	NDND
BL52749	0.4	4	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL52945	1.6	0	6.5	- 4	13	0	NDND	NDND
BL52945	0.4	22	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL52749	1.6	- 17	6.5	4	13	- 17	NDND	NDND
BL53308	0.4	- 35	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BJ07486	13	31	NDNDN	NDN	NDNDN	NDN	NDND	NDND
ZP46735	52	25	104	41	208	NDN	NDND	NDND
BH57098	52	33	104	70	NDNDN	NDN	74.9	2.52
BG11417	52	48	104	NDN	NDNDN	NDN	NDND	NDND
BH69918	26	13	52	21	NDNDN	NDN	NDND	NDND
BG48969	13	13	26	NDN	52	NDN	NDND	NDND
ZP40153	6.5	17	13	8	26	8	NDND	NDND
ZN81159	6.5	- 4	13	- 8	26	NDN	NDND	NDND
BH84540	52	- 4	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BG56265	52	0	208	NDN	NDNDN	NDN	NDND	NDND
ZP26037	26	0	104	- 50	NDNDN	NDN	NDND	NDND
BJ79222	52	- 17	208	NDN	NDNDN	NDN	NDND	NDND
BK61538	104	- 17	416	- 11	NDNDN	NDN	NDND	NDND
BE20390	52	22	208	53	NDNDN	NDN	192.	.869
BK22791	26	- 22	104	- 17	NDNDN	NDN	NDND	NDND
BE21039	26	24	104	37	NDNDN	NDN	NDND	NDND
BE50012	52	28	208	60	NDNDN	NDN	158.	1.05
BD09814	26	- 11	104	NDN	NDNDN	NDN	NDND	NDND
BK50562	26	- 11	104	NDN	NDNDN	NDN	NDND	NDND
BJ76365	26	20	104	NDN	NDNDN	NDN	NDND	NDND
BK01676	26	20	104	36	NDNDN	NDN	NDND	NDND
ZP12597	26	- 8	104	20	NDNDN	NDN	NDND	NDND
AH07870	6.5	0	26	46	NDNDN	NDN	NDND	NDND
BK63005	13	38	52	NDN	NDNDN	NDN	NDND	NDND
ZP49343	52	32	208	71	NDNDN	NDN	123.	1.42
ZP49110	104	32	416	86	NDNDN	NDN	206.	.851
BH47850	52	10	208	16	NDNDN	NDN	NDND	NDND
BE20532	52	- 8	208	- 12	NDNDN	NDN	NDND	NDND
BL06916	208	69	832	87	NDNDN	NDN	148.	1.14

Table IV. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
ZN25964	52	7	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BM14388	500	30	900	33	1400	0	NDND	NDND
BM14388	500	- 22	900	7	1400	NDN	NDND	NDND
BJ78501	26	4	104	56	NDNDN	NDN	95.0	1.78
BM18395	208	44	416	69	832	75	257.	.659
BK01845	26	58	NDNDN	NDN	NDNDN	NDN	22.6	7.51
BK50713	26	30	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BL48914	208	0	416	47	832	- 11	NDND	NDND
BM18402	52	11	416	33	NDNDN	NDN	NDND	NDND
BE21799	26	46	104	67	NDNDN	NDN	39.6	4.67
BE21799	26	26	104	73	NDNDN	NDN	64.7	2.86
BG32550	26	4	104	11	NDNDN	NDN	NDND	NDND
BH08773	26	7	104	NDN	NDNDN	NDN	NDND	NDND
BJ92403	26	4	104	NDN	NDNDN	NDN	NDND	NDND
BE11588	26	- 4	104	NDN	NDNDN	NDN	NDND	NDND
AH43214	52	- 7	208	15	NDNDN	NDN	NDND	NDND
ZN43766	52	7	208	11	NDNDN	NDN	NDND	NDND
BE20694	52	15	208	74	NDNDN	NDN	143.	1.29
BH65278	26	11	104	50	NDNDN	NDN	NDND	NDND
AG98492	26	43	104	58	NDNDN	NDN	62.0	2.24
BE71137	52	26	208	59	NDNDN	NDN	164.	.844
BJ58956	52	37	208	63	NDNDN	NDN	129.	1.07
BG62807	6.5	23	26	NDN	NDNDN	NDN	NDND	NDND
AG98536	125	74	500	90	NDNDN	NDN	80.7	1.72
BE20498	104	31	416	90	NDNDN	NDN	204.	.682
BE20783	26	23	104	68	NDNDN	NDN	69.7	1.99
BK72933	26	31	104	26	NDNDN	NDN	NDND	NDND
BH49872	37.5	40	150	43	NDNDN	NDN	NDND	NDND
BH58522	26	64	104	NDN	NDNDN	NDN	20.1	6.90
BK56733	268	NDN	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BK50713	26	15	104	NDN	NDNDN	NDN	NDND	NDND
BK74375	52	31	208	38	NDNDN	NDN	NDND	NDND
BE20407	26	15	104	47	NDNDN	NDN	NDND	NDND
ZP49325	26	4	104	4	NDNDN	NDN	NDND	NDND
AY97600	208	25	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BE11677	26	- 4	104	27	NDNDN	NDN	NDND	NDND
ZP46468	78	31	312	NDN	NDNDN	NDN	NDND	NDND
BE20970	26	- 8	104	41	NDNDN	NDN	NDND	NDND
BE21020	52	35	208	70	NDNDN	NDN	117.	1.66
BE21235	26	19	104	47	NDNDN	NDN	NDND	NDND
BE21280	26	15	104	81	NDNDN	NDN	64.4	3.03
BG81599	52	26	208	59	NDNDN	NDN	164.	1.12
ZP12391	52	38	208	79	NDNDN	NDN	95.1	1.94
BH89429	416	NDN	NDNDN	NDN	NDNDN	NDN	NDND	NDND
BJ30645	208	35	NDNDN	NDN	NDNDN	NDN	NDND	NDND
ZN29524	52	26	208	60	NDNDN	NDN	161.	1.14
BH47869	52	21	208	44	NDNDN	NDN	NDND	NDND
ZP26715	52	NDN	208	NDN	NDNDN	NDN	NDND	NDND
ZP40242	26	9	104	47	NDNDN	NDN	NDND	NDND
ZP40733	26	15	104	- 6	NDNDN	NDN	NDND	NDND
ZP47054	104	15	416	53	NDNDN	NDN	391.	.474
ZP50248	26	21	104	41	NDNDN	NDN	NDND	NDND

Table IV. (Continued)

BN	DOSE1	SUPPRES1	DOSE2	SUPPRES2	DOSE3	SUPPRES3	SD50	GI
BK50713	52	38	ND	ND	ND	ND	ND	ND
ZN43579	26	-4	ND	ND	ND	ND	ND	ND
AG75828	104	ND	ND	ND	ND	ND	ND	ND
BD29165	52	0	ND	ND	ND	ND	ND	ND
BG21744	52	32	ND	ND	ND	ND	ND	ND
BG22125	104	42	ND	ND	ND	ND	ND	ND
BE20354	52	24	104	38	ND	ND	ND	ND
BK74384	52	16	104	4	ND	ND	ND	ND
BE20925	52	12	208	58	ND	ND	179	ND
AX26820	52	20	208	28	ND	ND	ND	ND

Table V. Summary of the suppressive activity of selected compounds against *Leishmania (v.) braziliensis* in the hamster.

WEEKS POST TREATMENT

Treatment	IMK	% Wt. Change	WEEKS POST TREATMENT					
			1	2	3	4	6	8
			Av. lesion Size	Av. lesion Size	Av. lesion Size	Av. lesion Size	Av. lesion Size	Av. lesion Size
Vehicle Control	-	3	142	125	121	100	96	79
BL09186	832	6	43	40	19	45	66	52
	208	5	96	79	70	88	72	71
AG98536	416	4	23	17	10	12	24	31
	208	7	65	50	60	79	100	83
	104	5	75	83	63	108	75	96
	52	5	100	104	80	96	75	55
BE21020	416	-1	25	8	0	0	0	2
	208	4	33	37	33	22	43	75
	104	5	68	71	63	52	54	88
BE20407	416	0	21	12	4	4	8	12
	208	3	30	30	38	38	47	25
	104	4	63	66	52	49	53	44
BE20783	416	1	25	13	8	0	0	13
	208	2	20	28	28	24	31	40
	104	6	65	79	88	92	83	79
BE21280	208	0	31	29	22	34	10	55
	104	2	83	73	65	71	79	75
	52	5	79	92	78	83	75	79

Table V. (Continued)

WEEKS POST TREATMENT (CONT)

Treatment	IMK	% Wt. Change	1			2			3			4			6			8		
			Av. lesion Size	%	Supp	Av. lesion Size	%	Supp	Av. lesion Size	%	Supp	Av. lesion Size	%	Supp	Av. lesion Size	%	Supp	Av. lesion Size	%	Supp
BE20498	160	3	108	24		88	30		75	38		79	21		83	13		96	-21	
	80	2	129	9		108	13		125	-3		125	-25		82	15		79	0	
ZP12391	208*	-2	30	79		35	72		36	70		60	40		56	42		16	80	
	104	2	79	44		56	55		71	41		79	21		96	0		88	-11	
	52	2	113	21		94	25		100	17		92	8		88	9		79	0	
DMSO Control	-	3	129	-		129	-		104	-		129	-		100	-		121	-	
	832	-1	121	6		121	6		125	-20		108	16		100	0		100	17	
BN19990	208	3	133	-3		121	6		121	-16		108	16		96	4		100	17	
BN34778	832	4	104	19		117	10		117	-12		92	29		83	17		61	50	
	208	4	142	-10		129	0		133	-28		121	6		96	4		83	31	

* Toxic as indicated by death

Table VI. Summary of results obtained from studies on the comparative activity of selected compounds against both Leishmania (L.) donovani and Leishmania (V.) braziliensis.

WRNO	BN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY?
WR061250	AX26820	L. don.	im	26	-18	52	-28	104	21		
			po		-24		-32		-20		
		L. bras.	im	52	23	104	15	208	49		
			po		15		8		19		
WR211789	BK50713	L. don.	im	6.5	99	13	100	52	100	<6.5	
			po		99		100		100	<6.5	
		L. bras.	im	13	0	52	35	104	50	104.00	
			po		15		28		19		
WR211666	BG11417	L. don.	im	6.5	100	13	100	52	100	<6.5	
			po		100		100		100	<6.5	
		L. bras	im	13	12	52	25	104	ND		104
			po		13		45		ND		104
WR1122536	AG78374	L. don.	im	6.5	-2	13	2	52	61	<52	
			po	ND							
		L. bras.	im	26	15	52	48	104	70	58.00	104
			po	ND							
WR006023	AG98545	L. don.	im	13	-18	52	67	104	ND	41.70	104
			po		18		51		86	45.70	
		L. bras.	im	6.5	-8	26	-4	52	0		
			po		-24		-44		-48		
WR099029	AH16404	L. don.	im	6.5	4	13	9	26	9		
			po	ND							
		L. bras.	im	6.5	0	13	-4	26	33		
			po	ND							
WR249668	BJ92403	L. don.	im	13	17	52	91	104	100	24.30	
			po		-20		62		98	47.00	
		L. bras.	im	13	0	52	16	104	28		
			po		-36		-28		12		
WR223658	BG21744	L. don.	im	13	100	52	100	104	100	<13	
			po		100		100		100	<13	
		L. bras.	im	26	16	104	46	208	ND		208
			po		-28		23		62	175.00	

Table VI. (Cont'd.)

WRNO	BN	PARASITE	ROUTE	DOSE1	SUPPRESS1	DOSE2	SUPPRESS2	DOSE3	SUPPRESS3	SD50	TOXICITY?
WR223756	BG22125	L. don.	im	13	100	52	100	104	100	<13	
			po		100		100		100	<13	
		L. bras.	im	52	4	104	4	208	4		208
			po		-16		24		ND		
WR049577	AH07870	L. don.	im	6.5	14	13	26	26	34		
			po		-27		-16		-30		
		L. bras.	im	6.5	61	13	61	26	76	3.76	26
			po		0		-7		-3		
WR006007	AJ36812	L. don.	im	13	54	52	90	104	98	11.90	
			po	ND							
		L. bras.	im	13	7	52	37	104	61	79.80	
			po	ND							
WR006917	AH32668	L. don.	im	13	-13	52	2	104	-15		
			po	ND							
		L. bras.	im	52	0	104	3	208	-3		
			po	ND							
WR006014	AJ09575	L. don.	im	13	-4	52	1	104	84	82.30	
			po		10		25		48		
		L. bras.	im	26	24	104	11	208	19		
			po		-30		15		15		
WR007561	AJ09851	L. don.	im	13	16	52	40	104	44		
			po	ND							
		L. bras.	im	26	-19	104	-4	208	53		
			po	ND							
WR027795	BE20532	L. don.	im	6.5	3	13	-1	52	0		
			po		-4		14		10		
		L. bras.	im	13	7	52	11	104	11		
			po		-14		-7		14		
WR057023	BB18813	L. don.	im	13	11	52	56	104	99	49.40	
			po		-36		60		98	47.60	
		L. bras.	im	52	-7	104	19	208	-7		
			po		-21		-7		7		

Table VI. (Cont'd.)

WRNO	BN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY
WR053215	BB19758	L. don.	im	13	-14	26	-24	104	-25		
			po		-22		-27		-17		
		L. bras.	im	26	-4	104	0	208	11		
			po		-25		-2		-7		
WR006027	BE20112	L. don.	im	13	99	26	99	104	100	4.93	
			po		99		100		100	4.65	
		L. bras.	im	26	21	104	48	208	ND		208
			po		28		17		ND		208
WR027788	BE20318	L. don.	im	13	-17	26	-5	104	11		
			po		-35		-11		5		
		L. bras.	im	26	14	104	5	208	ND		208
			po		7		7		-3		
WR027792	BE20345	L. don.	im	13	-11	26	2	104	4		
			po		16		50		55	92.00	
		L. bras.	im	26	21	104	37	208	ND		208
			po		28		-3		14		
WR027793	BE20354	L. don.	im	6.5	15	13	50	52	77	26.90	
			po		26		28		77	29.90	
		L. bras.	im	13	30	52	47	104	67	59.30	
			po		7		44		72	61.00	
WR027794	BE20498	L. don.	im	13	22	26	30	104	84	53.20	
			po		20		40		68	52.80	
		L. bras.	im	26	37	104	46	208	60	133.00	208
			po		-15		15		53	199.00	
WR027796	BE20603	L. don.	im	ND							
			po	6.5	29	13	35	52	78	24.60	
		L. bras.	im	ND							
			po	13	9	52	4	104	26		
WR006881	BE20792	L. don.	im	6.5	25	26	13	52	46		
			po		21		28		20		
		L. bras.	im	6.5	2	26	42	104	54	77.70	
			po		-19		-26		-30		

Table VI. (Cont'd.)

WRNO	BN	PARASITE	ROUTE	DOSE1	SUPPRESS1	DOSE2	SUPPRESS2	DOSE3	SUPPRESS3	SD50	TOTALITY
WR027742	BE20925	L. don.	im	6.5	10	26	44	52	82	28.70	
			po		16		33		60	41.60	
		L. bras.	im	6.5	10	26	53	104	78	25.90	
			po		17		13		38		
WR027785	BE20943	L. don.	im	ND							
			po	6.5	26	13	27	52	52	48.50	
		L. bras.	im	ND							
			po	13	-35	52	4	104	58	96.10	
WR006877	ZN29695	L. don.	im	6.5	-37	13	17	52	72	35.60	
			po		-25		-13		76	38.20	
		L. bras.	im	13	13	52	41	104	71	65.00	
			po		-17		17		43		
WR027779	BE21039	L. don.	im	6.5	17	26	61	52	75	20.70	
			po		9		58		72	23.60	
		L. bras.	im	13	25	52	71	208	81	33.70	208
			po		20		57		75	44.30	
WR006020	BE20166	L. don.	im	6.5	7	26	15	52	57	23.60	
			po		1		14		47		
		L. bras.	im	13	16	52	42	104	32		
			po		-10		10		19		
WR027780	BE21084	L. don.	im	6.5	-1	26	65	52	83	18.80	
			po		-5		26		58	44.10	
		L. bras.	im	26	23	52	52	104	79	50.10	104
			po		-23		19		55	96.30	
WR007296	BE21511	L. don.	im	6.5	-41	13	-24	52	14		
			po		-7		1		26		
		L. bras.	im	13	-13	26	0	104	6		
			po		6		6		3		
WR006021	BE21799	L. don.	im	6.5	9	13	3	52	78	34.80	
			po		3		5		61	34.80	
		L. bras.	im	13	-12	26	-8	104	55	104.00	
			po		14		8		42		

Table VI. (Cont'd.)

WRNO	BN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY
WR052252	AT63681	L. don.	Im	13	3	52	22	104	-9		
			po	ND							
		L. bras.	Im	52	-15	104	-13	208	-13		
			po	ND							
WR254419	BL05848	L. don.	Im	6.5	74	13	93	52	99	4.16	
			po		64		83		98	4.47	
		L. bras.	Im	13	15	52	17	104	ND		104
			po		-19		-4		ND		104
WR254847	BL68705	L. don.	Im	6.5	79	26	89	52	85	1.71	
			po	ND							
		L. bras.	Im	13	35	52	43	104	56	79.60	
			po	ND							
WR007511	AJ15304	L. don.	Im	13	7	26	22	52	34		
			po	ND							
		L. bras.	Im	3.25	-9	13	-30	52	9		
			po	ND							
WR006561	AT56097	L. don.	Im	13	13	52	51	104	38	51.40	
			po	ND							
		L. bras.	Im	52	9	104	-4	208	13		
			po	ND							

Table VII. Summary of results obtained from selected oligonucleotide compounds* studied for suppressive activity against *Leishmania (L.) donovani* promastigotes in vitro.

<u>Compound</u>	<u>Percent Suppression</u>	<u>Compound</u>	<u>Percent Suppression</u>
LE001	7.81	LE002.06	J 910716
LE501	- 0.95	LE002.07	J 910716
LE002	5.17	LE001.01	J 910806
LE502	- 4.76	LE001.02	J 910806
LE003	-18.35	LE001.04	J 910808
LE503	-20.38	HBV040.01	J 910806
LE004	20.78	LE001QX	
LE504	32.07	LE002QX	
LE005	11.92	LE001SX	
LE505	14.14	LE002SX	
LE001	37.31	LE001.01H	
LE501	6.62	LE002.01H	
LE002	- 3.92	LE001.01Q	
LE502	-14.32	LE002.01Q	
LE003	- 4.04	LE001.01S	
LE503	6.36	LE002.01S	
LE004	43.56	LE001HX	
LE504	49.02	LE002HX	
LE005	18.46	LE001HY	
LE505	32.63	LE002HY	
LE502	34.4	LE001QY	
LE002.01 J 910415	20.8	LE002QY	
LE002.02 J 910415	25.5	LE001SY	
LE002.03 J 910416	22.2	LE001.01J	
LE002.04 J 910416	26.6	LE501.01J	
LE002.05 J 910716	26.3		
			21.6
			26.8
			91.4*
			14.3
			47.0
			38.8
			- 76.9**
			-222.5
			-179.2
			- 10.7
			17.4
			- 14.5
			- 34.9
			- 32.0
			- 27.5
			7.3
			8.3
			- 75.2
			32.8
			27.2
			16.0
			- 71.6
			- 2.4
			27.2
			53.0

* Based on triplicate cultures.

** Negative percent suppression indicates enhancement of parasite numbers.

Personnel Employed from this Contract

<u>Position and Title</u>	<u>Percent Effort</u>	<u>Length of Employment</u>
Research Coordinator II Virginia B. Waits	(Full-time; 100%)	09/28/90 - Present
Laboratory Technician II Mark J. Komoroski	(Part-time; 50%)	01/17/91 - 06/30/91
Laboratory Technician II Barbara L. Harris	(Full-time; Hourly, 100%)	09/23/91 - 12/23/91
Laboratory Technician II Barbara L. Harris	(Full-time; 100%)	01/09/92 - 06/30/93
Laboratory Technician II Barbara L. Harris	(Part-time; 90%)	07/01/93 - 08/11/93
Laboratory Technician II Barbara L. Harris	(Part-time; 50%)	08/12/93 - 09/27/93
Graduate Research Assistant Laura A. Lamb	(Part-time; 16½%)	07/01/92 - 09/30/92
Student Assistant (STUWK) Shannon L. Waits	(Part-time; 10%)	10/10/90 - 06/05/92

DEVELOPMENT OF *LEISHMANIA (VIANNIA) PANAMENSIS* LESIONS AND RELATIONSHIP OF NUMBERS OF AMASTIGOTES TO LESION AREA ON ANTIMONY-TREATED AND UNTREATED HAMSTERS

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ABSTRACT: Young adult (60–70-g) male golden hamsters (*Mesocricetus auratus*) each were injected intradermally at the dorsal base of the tail with 15×10^6 promastigotes of *Leishmania (Viannia) panamensis* (MHOM/PA/83/WR539), and progression and regression of subsequent lesions were evaluated for up to 17 wk postinfection (PI) as to area, weight, and number of amastigotes within lesions in untreated hamsters and in hamsters treated with meglumine antimoniate (Glucantime®). In untreated hamsters total area of lesion, weight, and numbers of amastigotes generally increased rapidly and concomitantly up to 3–4 wk PI. Amastigote numbers tended to decrease from 4 to 11 wk PI and subsequently the numbers of amastigotes within the lesions decreased rapidly, whereas relatively little change occurred in the area and weight of the lesions. Meglumine antimoniate treatment of cutaneous hamster lesions resulted in marked concomitant decrease in size of the lesions and numbers of amastigotes within the lesions examined 1 wk after treatment. Measurement of the area of cutaneous leishmanial lesions thus would appear to be a valid method of evaluating the efficacy of promising compounds against *L. panamensis* in hamsters when measurements are taken 3–5 wk after experimental infection and reflects the number of amastigotes present in the lesion.

The importance of the leishmaniasis to human health in many tropical and subtropical areas of the world, coupled with the need for better methods of prevention and treatment of these diseases, recently has stimulated considerable interest in the chemotherapy and immunology of these protozoan parasites. Both in vivo and in vitro test systems are important in the development of better therapeutic methods for *Leishmania*. The in vivo evaluation of new potential antileishmanial chemical and immunological agents often involves the comparison of parasite numbers and cutaneous leishmanial lesion areas in experimental animals subjected to various chemical compounds and immunological procedures (Neal, 1970; Walton et al., 1983; Liew et al., 1985).

Because of the complicated series of events leading to cutaneous leishmanial lesions induced by *Leishmania (Viannia) panamensis*, questions have arisen as to the relationship between lesion area and number of amastigotes present within the lesion and as to the value of lesion area as

an indicator of efficacy of potential antileishmanial chemical and immunological agents. Relatively little information is available on this subject.

The present studies were done to determine the relationship of age, weight, and area of cutaneous lesions to numbers of amastigotes within the lesions in hamsters following experimental infection with *L. panamensis*, and to determine the efficacy of meglumine antimoniate in reducing lesion area and weight and number of amastigotes within the lesion.

MATERIALS AND METHODS

Procedures for preparation of parasites, injection of hamsters, and evaluation of lesions were similar to those described previously (Childs et al., 1976; Wilson et al., 1979) except that in the present work, the base of the tail was the site of injection of the promastigotes. This site of inoculation was selected over the nose and the footpad because it allowed ease of measurement of the lesion in intractable hamsters.

Briefly, cutaneous leishmanial lesions caused by experimentally infecting hamsters with *L. panamensis* (MHOM/PA/83/WR539) were ground in sterile saline solution in a Ten Broeck tissue grinder, and amastigotes from this suspension were cultured in Schneider's *Drosophila* medium with 20% fetal calf serum (Childs et al., 1978; Hendricks et al., 1978). Suspensions of promastigotes for infection of the hamsters were obtained from eight-day third subcultures. Extensive experience has shown that promastigotes obtained under these circumstances consistently produce lesions of similar size. In preparation for injection, the hair was clipped from the dorsal base of each hamster's tail and,

Received 17 August 1990; revised 25 April 1991; accepted 25 April 1991.

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weekly during the experiment, a commercial depilatory agent was applied to the area. Each young (60–70-g) male golden hamster (*Mesocricetus auratus*) was injected intradermally with 15×10^6 promastigotes of *L. panamensis* near the base of the tail.

Lesion area was determined from either 4, 5, or 6 hamsters at specified intervals from 1 to 17 wk post-infection (PI). The single leishmanial lesion was excised from either 4, 5, or 6 hamsters at the same intervals, and area, lesion weight, and number of amastigotes in each lesion were determined. For determination of parasite numbers, the hamsters were killed and granulomatous tissue dissected from the deep epidermis, dermis, and subcutis was weighed and then ground in a measured volume of sterile saline solution in a Ten Broeck tissue grinder. The number of amastigotes in the lesion was determined by the procedure described previously (Hanson and Roberson, 1974).

In part of the experiment, each of 6 hamsters was given meglumine antimoniate (52 or 208 mg/kg/day, total dosage of 208 and 832 mg/kg) or vehicle (Hec-tween) intramuscularly (i.m.) on days 19–22 PI as indicated in Table II. The single cutaneous lesion from each of the 6 hamsters was measured with calipers, excised, and weighed, and number of amastigotes per lesion was determined at 1 wk after completion of treatment.

Because amastigote numbers were variable, the data were transformed using the log of $x + 1$ technique (Zar, 1984) prior to the analysis. The data were analyzed using an analysis of variance with a follow-up Tukey test. Following the analysis of the effect of meglumine antimoniate on the number of parasites, it was found that even after the logarithmic transformation the variances were significantly (Bartlett's test = 1.55, $P = 0.05$) heterogeneous. Data then were analyzed using a Kruskal-Wallis test. Multiple comparisons were made at a 0.15 experiment-size rate.

RESULTS

Cutaneous leishmanial lesions in untreated hamsters increased in area during the first 4 wk after infection and reached what was considered an optimal size for evaluation of the effect of experimental drugs by the third to the fourth wk PI (30–50 mm² at 3 wk and 50–200 mm² at 4 wk). The lesions subsequently maintained their size for up to 17 wk PI, at which time the experiments were terminated (Table I). Lesion weights also increased during the first 4 wk PI, with dense granulomas present during 3–11 wk PI (range, 61–884 mg of tissue). Numbers of amastigotes in the lesions increased rapidly during the first few weeks of infection, and significantly greater numbers of amastigotes were present in lesions by 3 wk PI ($\bar{x} = 14.0 \times 10^6$, $P < 0.05$) than were observed in lesions examined at 1 wk following infection ($\bar{x} = 1.4 \times 10^6$, Table I). Although mean numbers of amastigotes were not statistically different in lesions examined 4–

TABLE I. Mean area, weight, and number of amastigotes within cutaneous lesions of hamsters injected intradermally with 1.5×10^7 promastigotes of *Leishmania (Viannia) panamensis*.^a

Weeks postinfection	n	Lesion area (mean) (mm ²)	Mg tissue lesion (mean)	Mean number of amastigotes/lesion
1	6	20–40 (33)	10–53 (27)	1.40×10^6
3	6	30–50 (42)	61–137 (100)	14.00×10^6
4	6	50–200 (129)	78–394 (253)	9.70×10^6
5	6	75–150 (100)	149–452 (261)	7.70×10^6
7	6	40–150 (103)	112–280 (212)	2.60×10^6
9	5	50–125 (90)	100–262 (175)	0.99×10^6
11	5	75–150 (105)	117–884 (326)	1.20×10^6
14	4	75–125 (100)	56–233 (130)	0.56×10^6
17	4	(100)	ND	0.15×10^6

^a All measurements 100 mm². ND = not determined.

11 wk PI, there was a general trend toward a decrease in number, and by 14 and 17 wk PI the means of 0.56×10^6 and 0.15×10^6 amastigotes, respectively, were significantly lower than numbers observed at 3 wk ($P < 0.05$; Table I). When hamsters with cutaneous lesions were treated with meglumine antimoniate at 19–22 days PI, both lesion area and total number of amastigotes per lesion were noted to decrease. The lesion area was suppressed by 55% in hamsters treated with a total dosage of 208 mg of antimony (Sb) over a 4-day period and by 76% in those treated with a total dosage of 832 mg of Sb as meglumine antimoniate. Total numbers of amastigotes within lesions were decreased by 81% and 96% in hamsters treated with 208 and 852 mg of Sb as meglumine antimoniate, respectively (Table II; $P < 0.05$ at 852 mg/kg).

DISCUSSION

The efficacy of vaccination procedures and promising antileishmanial drugs against several species of cutaneous *Leishmania* have been evaluated by comparing lesion areas in groups of untreated and treated experimental animals (Neal, 1970; Walton et al., 1983; Liew et al., 1985). Due to the complicated series of events that occurs between the infection of the host by the amastigote and the development of the cutaneous lesion, the question arises as to whether lesion area accurately reflects change in number of amastigotes present in the lesion. Therefore, it becomes of some importance to relate the area and weight of the cutaneous lesions experimentally to the numbers of amastigotes present in untreated as well as treated hamsters and to study

TABLE II. Effect of meglumine antimoniate on mean area of hamster cutaneous leishmanial lesions and mean numbers of amastigotes at 7 days after treatment.*

Treatment	Total dosage (mg/kg)	Lesion area (mean) (mm ²)	% Suppression of lesion area	Weight of tissue/lesion (mean) (mg)	Total amastigotes/lesion	% Suppression of amastigotes
Vehicle	—	75–150 (129)	0	149–452 (253)	9.7×10^4	0
52 mg/kg/day	208	20–125 (58)	55	26–222 (92)	2.8×10^4	81
208 mg/kg/day†	832	20–40 (30)	76	16–29 (25)	0.4×10^4	96

* n = 6, each treatment.

† Significantly different from vehicle (Bartlett's test; $P < 0.05$).

this relationship at intervals during the progression and regression of the lesions.

Prior investigators have reported transient experimental cutaneous leishmanial lesions in hamsters infected with *L. panamensis* for 4–5 wk PI, followed by a decrease in mean lesion size suggesting the onset of acquired immunity and self-cure (Neal and Hale, 1983). The results obtained in the present study differ from those of the previous investigators as regards persistence of lesions. In our studies, numbers of amastigotes increased during the first 3 wk and lesion size and weight increased during the first 4 wk following infection of hamsters with promastigotes of *L. panamensis*. Subsequently, the mean lesion area and weight remained approximately the same for the next several weeks. However, the numbers of amastigotes tended to decrease during this time and by 14–17 wk they had decreased markedly. In order to evaluate the efficacy of chemotherapy on cutaneous lesions, our data suggest that lesion area should be determined during the period of the infection when the lesions and numbers of amastigotes are increasing and when the host immune response is having a minimal effect on lesion area. Based on the observations reported herein, we suggest that potential antileishmanial drugs against *L. panamensis* in hamsters should be evaluated during the first 3 or 4 wk of the infection when both lesion area and numbers of amastigotes are increasing. This is the procedure currently used in this laboratory for evaluation of potential antileishmanial drugs. Any difference in lesion area or number of amastigotes in treated compared to control animals should be due to the experimental treatment rather than to host response. However, the host response appears to be minimal for up to 17 wk PI with regard to lesion reduction, as no change in lesion size or weight was seen 4–17 wk PI.

It is necessary also to determine whether the

decrease in lesion area following therapy reflects a decrease in numbers of amastigotes as a result of the killing of the parasites by the drug used. The data presented here confirm that meglumine antimoniate therapy results in a marked decrease in numbers of amastigotes in the lesions and a concomitant reduction in lesion area when the drug is administered at 19–22 days PI. Thus, lesion area in this model is an accurate indicator of antileishmanial drug efficacy when evaluated at 3–4 wk PI.

As a result of these observations, the test system utilized in our laboratory with the hamster and *L. panamensis* is to allow the infection to progress for 19 days, administer treatment at 19–22 days PI, and determine the lesion areas of all hamsters 1 wk after completion of treatment. This protocol allows completion of the experiment during the first 4 wk of infection when lesion size and number of amastigotes are increasing rapidly and concomitantly.

The decrease in number of amastigotes as the age of the lesion increases has implications for diagnosis of cutaneous leishmaniasis in humans. Microscopic and cultural demonstrations of the presence of amastigotes in cutaneous lesions are the only methods of making a positive diagnosis of cutaneous leishmaniasis. Assuming that the same relationship between lesion age and amastigote number occurs in human beings as in hamsters, the low numbers of parasites in lesions of long duration could explain the difficulty often experienced in attempting to demonstrate amastigotes microscopically in long-standing human infections.

ACKNOWLEDGMENTS

This work was supported by the U.S. Army Medical Research and Development Command through Contract No. DAMD 17-85-C-5012. Opinions, interpretations, conclusions, and rec-

ommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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